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Research article

Evaluation of scoring atopic dermatitis (SCORAD) and scratching behavior in BALB/c mice treated with house dust mite immunotherapy

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Abstract

Allergen specific immunotherapy controls the reaction and builds immunological tolerance by giving an allergen in escalating doses. Research on immunotherapy in atopic dermatitis (AD) mouse model offers a new perspective on the approach of treatment in AD. This was an experimental study of 33 male BALB/c mice, 6-8 weeks old, divided into 3 groups (control, AD model, and house dust mite/HDM immunotherapy). The mice received spray and patch containing allergen extract of *Dermatophagoides pteronyssinus*. Immunotherapy was injected subcutaneously in increasing doses. The evaluation of SCORAD and scratching behavior were observed at the end of the treatment on day 93. The SCORAD of the model group that received HDM allergen had a mean of 1.27 ± 0.467 and the immunotherapy group had a mean of 0.36 ± 0.505. There were significant differences between the groups. The model group had a mean of 5.18 ± 4.119 and the immunotherapy group had mean of 1.55 ± 1.293. The statistical analysis showed that there were significant differences between control group and model group, as well as model group and immunotherapy group. Interobserver agreement was assessed and showed substantial agreement for SCORAD ($\kappa = 0.613$ and $p < 0.001$) and scratching evaluation ($\kappa = 0.714$ and $p < 0.001$). House dust mite immunotherapy significantly reduced SCORAD and scratching behavior in BALB/c mice compared to placebo groups.

Keywords: Atopic dermatitis, BALB/c mice, House dust mites, Immunotherapy, Tropical disease

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INTRODUCTION

House dust mites (HDM) are the most prevalent aeroallergens associated with atopic disease, with some countries reporting high percentages of HDM sensitivity (Tham et al., 2016). Atopic reactivity to HDM affects the skin, eyes, upper and lower airways, and occasionally systemic circulation. Sensitization to mite allergens is common in atopic dermatitis (AD), with IgE sensitization to the mite allergen present in 95% of patients, compared to 42% in asthma patients and 17% in healthy controls (Miller, 2019). The number of HDM sensitization detected with skin prick test among AD patients in Surabaya, Indonesia was 63.3% according to a study (Nugroho et al., 2022). Warm temperatures and high humidity levels enhance the fast growth of house dust mites. As a result, HDM populations will be more common in tropical areas with warm temperatures throughout the year and much more so in houses with inadequate room ventilation and a high level of humidity (70-80%). *Dermatophagoides pteronyssinus* and *Dermatophagoides farinae* are two HDM species that are frequently found in Indonesia and have been linked to atopic disease (Anggraeni et al., 2022; Sarwar, 2020).

Allergen specific immunotherapy can be taken into consideration as an etiological treatment of AD. Essentially, allergen specific immunotherapy gives an allergen in escalating doses in order to control the reaction and build immunological tolerance. Allergen specific immunotherapy (AIT) triggers a switch from Th2 to Th1 immune response pattern, a reduction in mast cell mediator release, and induce the blocking antibodies IgG4 (Ridolo et al., 2017). When the right doses of allergens are delivered, AIT is successful, offering a clinical improvement in allergic rhinitis and asthma, including decreased symptoms, less medication use, and improved quality of life, with a long-lasting result even after therapy has stopped (Tang, 2020). According to a study, HDM immunotherapy was effective in relieving respiratory symptoms of allergies and decreased the number of days/weeks free from allergy symptoms among children with airway symptoms of allergy and AD, urticaria or gastrointestinal disorders as comorbidities (Endaryanto, 2019).

Various kinds of mouse models have been used in studies of AD. Mouse models of AD present a model for better understanding disease pathophysiology and establishing new therapies, but they have limitations in accurately represent human AD as different mouse strains exhibit different immune responses (Lee et al., 2010; Martel et al., 2017). A study of immunotherapy in AD mouse model using monomeric allergoid created by succinylating ovalbumin (OVA) showed alleviation of skin lesions and enhanced immunological and histological aspects compared to non-succinylated OVA and placebo (Shershakova et al., 2015). Data obtained from research on immunotherapy in the AD mouse model offers a new perspective on the approach to treatment in AD. This study aimed to understand the effects of HDM immunotherapy by observing the severity of skin lesion and scratching behavior in AD mouse model.

MATERIALS AND METHODS

Thirty-three male BALB/c mice, 6-8 weeks old, were divided into 3 groups, (1) control group, (2) AD model group, and (3) HDM immunotherapy group. The control group received spray and patch with NaCl 0.9% as placebo. The model and immunotherapy group received spray and patch containing Dermatophagoides pteronyssinus (Der p) allergen extract with a dose of 100 µg for patch and 10-6 µg for spray. The Der p allergen used in this study was produced by Teaching Industry Allergen by Dr.Soetomo Hospital-Universitas Airlangga, Surabaya, Indonesia. The spray was given to the mice from day 1 to 93 with nebulizers in closed chambers. Immunotherapy was injected to the neck of the mouse subcutaneously. The immunotherapy group received HDM immunotherapy injection containing Der p extract with increasing dose of 0.1 µg (on day 15, 18, 21, and 24), 1 µg (on day 27, 30, 33, and 36), 10 µg (on day 39, 42, 45, and 48), and 100 µg (on day 51, 52, 57, and 60) in 100 µL phosphate-buffered saline (PBS). Immunotherapy was given every 3 days, 4 times for each dose. Mice in control and model group received PBS injection as placebo. The model and immunotherapy group received HDM allergen patch for 1 week before starting the immunotherapy to induce AD-like lesions. The timeline for the patch, spray and immunotherapy for the mice is shown in Figure 1.

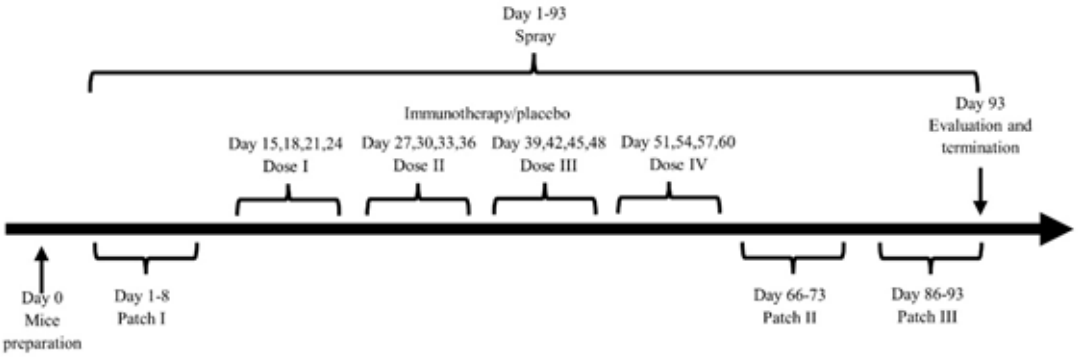

Figure 1 Timeline of treatment and immunotherapy for the mice.

The evaluation of SCORAD and scratching behavior was observed at the end of the treatment on day 93 by two independent observers. The SCORAD was evaluated on the back of each mouse where the patch was put before examination. Photos of the mouse's back were also taken for documentation. The SCORAD examination was based on the degree of erythema/hemorrhage, scarring/dryness, edema, and excoriation/erosion with a score of 0 to 3 according to the severity (0=absent, 1=mild, 2=moderate, 3=severe). A five-minute video of each mouse was recorded with a standard phone camera (1280x720 pixels, 29 frames per second) to observe the scratching behavior. The recording was made on each mouse in a specially made cage for recording (Figure 2). Two researchers counted the scratching bouts individually according to the counting method of scratching bout. A bout of scratching was counted starting from lifting the hind paw to the area to be scratched until the paw was placed back down or the mouse started grooming. Data were collected and analyzed with SPSS software (ver. 26, IBM) and Prism (ver. 8, GraphPad) for data visualization.

The nonparametric Kruskal-Wallis test was used for statistical comparative analysis with the Mann-Whitney test as post hoc analysis because the data were not normally distributed. Agreement between researchers was determined for every score item independently using Cohen's Kappa coefficient (κ). Statistical significance was determined by $p < 0.05$ and $\kappa = 0.41$-0.60 indicates moderate agreement, while 0.61-0.80 substantial agreement (Warrens, 2015). This study had received ethical clearance from The Ethical Committee of Faculty of Veterinarian Medicine Universitas Airlangga (No. 2.KE.11.09.2021).

Figure 2 The mouse was recorded for observation of scratching behavior in a cage specially made for recording.

RESULTS

Eleven mice from each group were evaluated after 3 months of treatment. The SCORAD and scratching behavior were counted. The back of the mice from each group are shown in Figure 3. Interobserver agreement was assessed with $\kappa=0.613$ and $p<0.001$ which means substantial agreement. The control group did not show any AD-like lesions in all mice. Meanwhile, the SCORAD of the model group that received HDM allergen had a mean of 1.27 ± 0.467 and the immunotherapy group had a mean of 0.36 ± 0.505. There were significant differences between AD model and immunotherapy group ($p = 0.001$), control with AD model group ($p = 0.000$), and control with immunotherapy group ($p = 0.031$) which can be observed in Figure 4a.

Scratching behavior evaluation from all groups of mice revealed that despite zero SCORAD in control group, the group had scratching bouts with mean of 0.82 ± 1.079. The model group had mean of 5.18 ± 4.119 and the immunotherapy group had mean of 1.55 ± 1.293. The agreement between observers for scratching evaluation showed substantial agreement with $\kappa = 0.708$ and $p <0.001$. The statistical analysis showed that there were significant differences between control group and model group ($p = 0.001$), as well as model group and immunotherapy group ($p = 0.010$). Meanwhile, the difference between the control group and the immunotherapy group was not significant (Figure 4b).

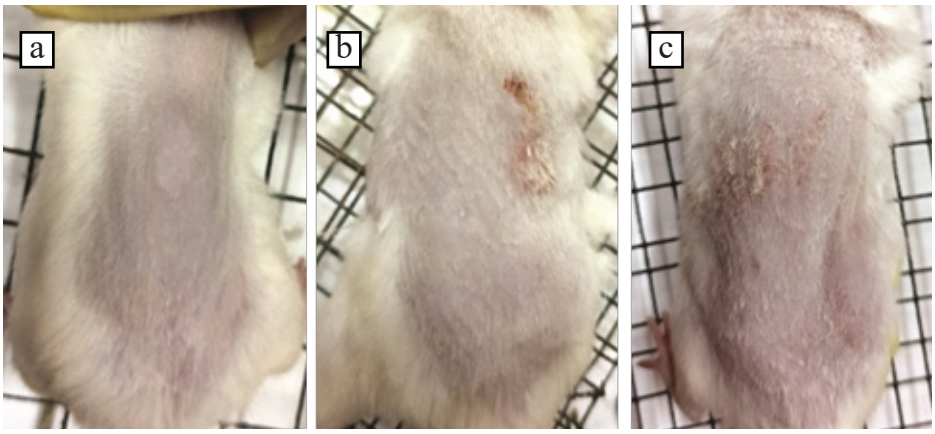

Figure 3 SCORAD evaluation on the back of the mice in each group. (a) Control group mouse shows no lesion, (b) model group mouse shows erythematous and hemorrhagic lesion, and (c) immunotherapy group shows mild erythematous and dry skin.

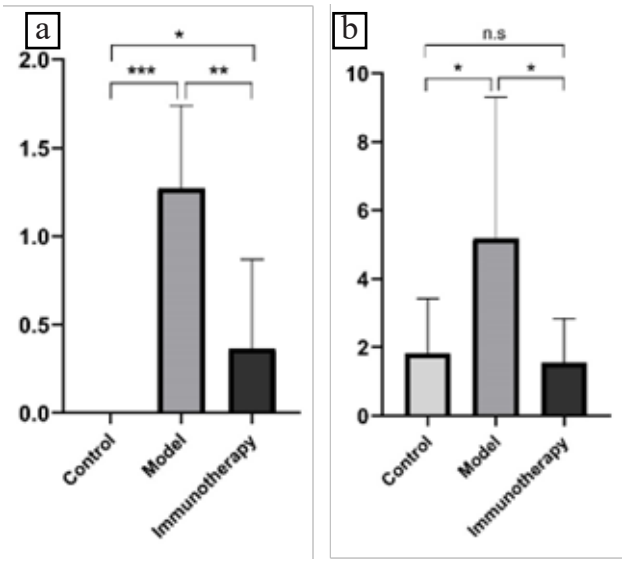

Figure 4 The comparison of SCORAD and scratching behavior between groups. (a) SCORAD comparation revealed significant differences between groups and (b) scratching bouts between groups were significantly different except between control and immunotherapy group. Results are presented as mean ± SD (Note: * $p < 0.05$; ** $p < 0.01$; *** $P < 0.001$; n.s not significant).

DISCUSSION

The most common aeroallergen linked to atopic disease in Asia is HDM, followed by cockroach, mold, animal dander, grass, and tree pollen. Environmental or indoor allergens, such as dust mites, often do not fluctuate significantly in South-east Asian nations with stable year-round tropical temperatures (Tham et al., 2016). Warm temperatures and high humidity levels increase the rapid growth of house dust mites (Sarwar, 2020). Most of the Asian atopic population can be deemed to have dust mite sensitivity. According to data from the majority of the Asian nations, the rates of sensitized atopic persons could reach up to 80% (Tham et al., 2016). The main approach

to treating an existing atopic disease is symptom management and allergen avoidance, which could be a real challenge in a tropical country with a large house dust mite population.

Atopic dermatitis patients who undergo patch testing with mite extract typically develop eczematous skin lesions, especially if their skin lesions are distributed in an area that is exposed to the air. This suggests that aeroallergens can induce the disease by coming into contact with the skin (Miller, 2019). In AD patients, tight junctions and skin barrier function were damaged as a result of mite allergen enzymatic activity (Bumbacea et al., 2020). Mite allergens have an impact on human keratinocytes, causing them to release pro-inflammatory and pro-Th2 cytokines and activate the NLR family pyrin domain containing 3 (NLRP3) inflammasome in the innate immune system (Miller, 2019). The activation of proteinase activated receptor 2 (PAR2) in epidermal keratinocytes and dermal nerves as a result of protease activity plays a significant role in the development of histamine associated pruritus (Bumbacea et al., 2020).

One such breakthrough treatment in atopic diseases is allergen-specific immunotherapy (AIT), which targets the cause of IgE-mediated allergic diseases and was first used in clinical practice more than a century ago (Bae et al., 2013; Caminiti et al., 2020). Immunotherapy is a disease-modifying treatment that is subjected to outdo the previous therapeutic approach such as allergen avoidance and symptom management using moisturizers, corticosteroids, and targeted disease-modifying drugs. A study had described AIT as the only causal treatment by providing gradually increasing doses of the allergen to produce hyposensitization, hence lowering symptoms upon natural exposure to the allergen and lowering the need for drug therapy (Alvaro-Lozano et al., 2020; Caminiti et al., 2020; Giannetti et al., 2020).

The effect of AIT appears to be associated with altered monocyte, B-cell, and T-cell functions. In dendritic cells, AIT increases the formation of natural regulatory T-cells (nTregs) and induced regulatory T-cells (iTregs) by releasing IL-12, IL-10, and IL-27. The immune response can be switched from an allergic Th2 to a Th1 response by specific Tregs and B-regulatory cells. Tregs also suppress the number and activity of mucosal mast cells, basophils, and eosinophils. AIT increases specific IgG4 and decreases specific IgE to allergens via Treg cells (Giannetti et al., 2020). In a study about mouse model of AD, a hapten was repeatedly painted on the skin, which caused the immune response to change from a Th1 to a Th2-dominated response as well as the development of high serum IgE levels, epidermal hyperplasia, and dermal mast cell infiltration, all of which are believed to be distinctive features of human AD. The different mice strains will result in different immune responses. For instance, the C57BL/6 strain mice promotes the Th1 response, whereas the BALB/c strain mice favors the Th2 response (Lee et al., 2010).

In several cases of allergic rhinitis, asthma, and venom allergies, the two traditional routes of AIT known as subcutaneous (SCIT) and sublingual (SLIT) have been used successfully for many years (Alvaro-Lozano et al., 2020). The use of the HDM AIT in allergic asthmatic children and adults has shown positive clinical results (Tang, 2020). The Indonesia-based study provides convincing evidence for the therapeutic advantages and cost-saving advantages of HDM SCIT in children with allergic rhinitis (Endaryanto and Nugraha, 2021). A randomized controlled trial of HDM immunotherapy in

children with rhinosinusitis also showed improvements in patient's immunity, sleep disorder, and quality of life (Putera et al., 2021). Various human researches examining the potential role of AIT in AD have been carried out (Alvaro-Lozano et al., 2020). The effectiveness of AIT for people with AD has generated debate due to conflicting clinical data and variations in immune response of AD models.(Bae et al., 2013) Several systematic reviews reported of conflicting results with limited evidence and a high potential for bias in the studies (Bae et al., 2013; Tam et al., 2016). The research of immunotherapy in AD models had uncertain results due to some limitations in AD models (Sugita and Akdis, 2020). This study was intended to better understand the effect of immunotherapy on AD.

In this study, the clinical score using SCORAD and the scratching behavior of AD mouse models were evaluated after a series of immunotherapy administered to the mouse. The skin lesions that formed on the back of the mouse were evaluated for the degree of erythema/hemorrhage, scarring/dryness, edema, and excoriation/erosion. Each symptom was assigned a score of 0 to 3, with a score of 3 indicating the most severe symptom. The maximum score for this assessment is 12. A previous study using *Dermatophagoides farinae* extract showed lower clinical symptoms in the immunotherapy group of NC/Nga mice. However, the result was not statistically significant at the end of the evaluation on the eighth week compared to placebo (Shin et al., 2018). The evaluation of SCORAD in this study found a significant difference between immunotherapy, AD models, and control group. The result shows that the effect of immunotherapy in mice can be seen on the lower SCORAD compared to the mice in AD model group that did not receive immunotherapy. Another clinical evaluation of the mice was scratching behavior. The effect of immunotherapy in reducing the itchy sensation from the AD-like lesion formed on mice can be found in this study. The scratching bouts in immunotherapy group was lower than AD model group ($p<0.05$), meanwhile there was no significant difference in immunotherapy and control group. Scratching behavior is described as a motor behavior that indicates an itchy sensation. Scratching in response to itching is also a sign of chronic inflammatory skin diseases such as AD. Scratching is frequently the only insight into itch sensation in modern itch research, and thus it is employed as an important, objective behavioral indication of increased or decreased itch, based only on scratching incidence or the number of scratching bouts (Wimalasena et al., 2021).

In mice, scratching can also be considered as a part of self-grooming (Estanislau et al., 2019; Maze Engineers, 2019). It is evident from clinical reports that the itch sensation carried on by different conditions varies in severity and quality, thus results in different incidence and quality of scratching. To further evaluate the complexity of the scratching behavior of mice, high-speed video recording with as much as 500 frames per second and a special program to locate the position of the paws are required (Wimalasena et al., 2021). Providing an environment with allergen-free for the mice was a challenge in this study, as HDM is commonly found in tropical countries which makes it difficult to avoid. This can be seen from the control group, even though there were no lesions on the skin, scratching behavior was still observed in some of the mice. There was a possibility of HDM exposure from the environment where the mice were kept which affected the clinical evaluations.

CONCLUSIONS

House dust mite immunotherapy significantly reduced SCORAD and scratching behavior in BALB-c mice compared to placebo group.

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AUTHOR CONTRIBUTIONS

SA: study design, data collection and interpretation, writing the first draft and final approval of the manuscript. DCT: study design, data collection, analysis and interpretation, review, revising and final approval of the manuscript. AE: study design, data collection, interpretation, review, revising and final approval of the manuscript. CRS: study design, funding acquisition, data interpretation, review, revising and final approval of the manuscript.

CONFLICT OF INTEREST

The authors declare no conflicts of interest regarding this publication.

REFERENCES

Alvaro-Lozano, M., Akdis, C.A., Akdis, M., Alviani, C., Angier, E., Arasi, S., Arzt-Gradwohl, L., Barber, D., Bazire, R., Cavkaytar, O., Comberiati, P., Dramburg, S., Durham, S.R., Eifan, A.O., Forchert, L., Halken, S., Kirtland, M., Kucuksezer, U.C., Layhadi, J.A., Matricardi, P.M., Muraro, A., Ozdemir, C., Pajno, G.B., Pfaar, O., Potapova, E., Riggioni, C., Roberts, G., Rodríguez del Río, P., Shamji, M.H., Sturm, G.J., Vazquez-Ortiz, M., 2020. Allergen immunotherapy in children user's guide. Pediatr. Allergy. Immunol. 31, 1-101.

Anggraeni, S., Paramitha, B.A., Triadi Nugroho, W., Umborowati, M.A., Damayanti, Endaryanto, A., Rosita Sigit Prakoeswa, C., 2022. Clinical relevance of house dust mites as causative factor of atopic dermatitis in Surabaya-Indonesia. In Proceedings of the 5th international conference on sustainable innovation, pp. 41-45.

Bae, J.M., Choi, Y.Y., Park, C.O., Chung, K.Y., Lee, K.H., 2013. Efficacy of allergen-specific immunotherapy for atopic dermatitis: A systematic review and meta-analysis of randomized controlled trials. J. Allergy. Clin. Immunol. 132, 110-117.

Bumbacea, R., Corcea, S., Ali, S., Dinica, L., Fanfaret, I., Boda, D., 2020. Mite allergy and atopic dermatitis: is there a clear link? (Review). Exp. Ther. Med. 20, 3554-3560.

Caminiti, L., Panasiti, I., Landi, M., de Filippo, M., Olcese, R., Ciprandi, G., Vernich, M., Carelli, F., Votto, M., Barberi, S., 2020. Allergen immunotherapy in atopic dermatitis: Light and shadow in children. Pediatr. Allergy. Immunol. 31, 46-48.

Endaryanto, A., 2019. Effects of atopic dermatitis, urticaria and gastrointestinal disorders as comorbidity in clinical improvement and parent satisfaction in children with moderate-persistent airway symptoms of allergy who get house dust allergen immunotherapy. Berk. Ilmu. Kesehat. Kulit. Kelamin. 31, 111-122.

Endaryanto, A., Nugraha, R.A., 2021. Indonesia-based study of the clinical and cost-saving benefits of subcutaneous allergen immunotherapy for children with allergic rhinitis in private practice. Cells. 10, 1841.
Estanislau, C., Veloso, A.W.N., Filgueiras, G.B., Maio, T.P., Dal-Cól, M.L.C., Cunha, D.C., Klein, R., Carmona, L.F., Fernández-Teruel, A., 2019. Rat self-grooming and its relationships with anxiety, dearousal and perseveration: Evidence for a self-grooming trait. Physiol. Behav. 209, 112585.
Giannetti, A., Ricci, G., Procaccianti, M., Santoro, A., Caffarelli, C., 2020. Safety, efficacy, and preventive role of subcutaneous and sublingual allergen immunotherapy for the treatment of pediatric asthma. J. Asthma. Allergy. 13, 575-587.
Lee, K.S., Jeong, E.S., Heo, S.H., Seo, J.H., Jeong, D.G., Choi, Y.K., 2010. A novel model for human atopic dermatitis: application of repeated DNCB Patch in BALB/c Mice, in comparison with NC/Nga mice. Lab. Anim. Res. 26, 95-102.
Martel, B.C., Lovato, P., Bäumer, W., Olivry, T., 2017. translational animal models of atopic dermatitis for preclinical studies. Yale. J. Biol. Med. 90, 389–402.
Maze Engineers, 2019. Mouse Ethogram: Self-Grooming Behavior. Available online: https://conductscience.com/maze/mouse-ethogram-self-grooming-behavior/ (Accessed on 30 May, 2022).
Miller, J.D., 2019. The role of dust mites in allergy. Clin. Rev. Allergy. Immunol. 57, 312-329.
Nugroho, W.T., Ervianti, E., Zulkarnain, I., Nurul Hidayati, A., Damayanti, Utomo, B., Rosita Sigit Prakoeswa, C., 2022. Characteristics of atopic dermatitis patients who underwent skin prick test. Berk. Ilmu. Kesehat. Kulit. Kelamin. 34, 11-14.
Putera, A.M., Hikmah, Z., Endaryanto, A., Irwanto, Maramis, M.M., 2021. The role of house dust mite immunotherapy in Indonesian children with chronic rhinosinusitis allergy: A randomized control trial. Heliyon. 7, e06510.
Ridolo, E., Martignago, I., Riario-Sforza, G.G., Incorvaia, C., 2017. Allergen immunotherapy in atopic dermatitis. Expert. Rev. Clin. Immunol. 14, 61–68.
Sarwar, M., 2020. House dust mites: ecology, biology, prevalence, epidemiology and elimination. In: Pacheco, G.A.B., Kamboh, A.A. (Eds.), Parasitology and microbiology research. IntechOpen, London.
Shershakova, N., Bashkatova, E., Babakhin, A., Andreev, S., Nikonova, A., Shilovsky, I., Kamyshnikov, O., Buzuk, A., Elisyutina, O., Fedenko, E., Khaitov, M., 2015. Allergen-specific immunotherapy with monomeric allergoid in a mouse model of atopic dermatitis. PLoS One. 10, e0135070.
Shin, J.U., Kim, S.H., Noh, J.Y., Kim, J.H., Kim, H.R., Jeong, K.Y., Park, K.H., Lee, J., Chu, H., Lee, J.H., Yong, T.S., Park, J.W., Lee, K.H., 2018. Allergen-specific immunotherapy induces regulatory T cells in an atopic dermatitis mouse model. Allergy. 73, 1801-1811.
Sugita, K., Akdis, C.A., 2020. Recent developments and advances in atopic dermatitis and food allergy. Allergol. Int. 69, 204-214.
Tam, H.H., Calderon, M.A., Manikam, L., Nankervis, H., Núñez, I.G., Williams, H.C., Durham, S.R., Boyle, R.J., 2016. Specific allergen immunotherapy for the treatment of atopic eczema: A cochrane systematic review. Allergy. 71(9), 1345-1356.
Tang, R. bin, 2020. House dust mite-specific immunotherapy alters the natural course of atopic March. J. Chin. Med. Assoc. 83, 109-112.
Tham, E.H., Lee, A.J., van Bever, H., 2016. Aeroallergen sensitization and allergic disease phenotypes in Asia. Asian. Pac. J. Allergy. Immunol. 34, 181-189.
Warrens, M.J., 2015. Five ways to look at Cohen's Kappa. J. psychol. psychother. 5, 1000197.
Wimalasena, N.K., Milner, G., Silva, R., Vuong, C., Zhang, Z., Bautista, D.M., Woolf, C.J., 2021. Dissecting the precise nature of itch-evoked scratching. Neuron. 109, 3075-3087.

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